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MINIREVIEW

The Bacterial Enhancer-Dependent σ^{54} (σ^{N}) Transcription Factor

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The initiation of transcription is a complex process involving many different steps. These steps are all potential control points for regulating gene expression, and many have been exploited by bacteria to give rise to sophisticated regulatory mechanisms that allow the cell to adapt to changing growth regimens. Before they can transcribe from specific DNA promoter sequences, bacterial core RNA polymerases (with subunit composition $\alpha_2\beta\beta'$) must combine with a dissociable sigma subunit (σ) to form RNA polymerase holoenzyme ($\alpha_2\beta\beta'\sigma$). Since the discovery of σ factors (6), it has become clear that these proteins are central to the function of the RNA polymerase holoenzyme. The reversible binding of alternative σ factors allows formation of different holoenzymes able to distinguish groups of promoters required for different cellular functions. In addition to double-strand DNA promoter recognition and binding, σ proteins are closely involved in promoter melting (e.g., references 31, 36, 49, 51, 74, 76, 128), inhibit nonspecific initiation, are targets for activators, and control early transcription through promoter clearance and release from RNA polymerase (48, 49, 53). Here we describe the functioning of the bacterial σ^{54}-RNA polymerase that is the target for sophisticated signal transduction pathways (103) involving activation via remote enhancer elements (5, 95).

Based on structural and functional criteria, the different σ factors identified in bacteria can be grouped in two classes, one of which has a single member, σ^{54}. Many σ factors belong to the σ^{70} class, the major σ factor which is involved in expression of most genes during exponential growth (72). σ^{54} (also called σ^{N}) differs both in amino acid sequence and in transcription mechanism from the σ^{70} class (80). Despite the lack of any significant sequence similarity, both types of σ bind the same core RNA polymerase. Nonetheless, they produce holoenzymes with different properties.

With the recognition that the σ^{54} protein represented an entirely new class of σ factor, what had once been regarded as an aspect of transcription restricted to higher organisms became a well-established feature of certain bacterial regulatory systems, particularly those associated with nitrogen metabolism. Activation of σ^{54}-RNA polymerase employs specialized bacterial enhancer-binding proteins whose activating function requires nucleotide hydrolysis (94, 96, 122) (Fig. 1). In this system, initiation rates are controlled via regulation of the DNA melting step that is necessary for establishing the open promoter complex (85, 94, 97). Bacterial enhancer-dependent transcription can be studied with just two purified proteins (an activator and the σ^{54}-RNA polymerase holoenzyme) and the appropriate DNA template, facilitating progress in understanding mechanistic aspects of σ^{54} functioning. Below we review the biology and biochemistry of the σ^{54}-RNA polymerase.

OCCURRENCE AND FUNCTION OF σ^{54}

Although σ^{54} was originally recognized in the enteric bacteria, it is now clear that σ^{54} is widely distributed among the bacteria. The role of σ^{54}-RNA polymerase, historically in regulation of nitrogen metabolism and subsequently in many other biological activities, is well established in many proteobacteria (80). Genes encoding σ^{54} have also been cloned from the gram-positive *Bacillus subtilis*, where σ^{54} is involved in utilization of arginine and ornithine (43) and transport of fructose (29), and from *Planctomyces limnophilus* (68). Furthermore, genome sequencing projects have revealed open reading frames potentially encoding σ^{54} in diverse bacteria such as an extreme thermophile (30, 105), obligate intracellular pathogens (58, 102), spirochetes (37, 38), and green sulfur bacteria (104). However, complete genome sequences have also revealed the absence of σ^{54} in a diverse range of bacteria including the high-G+C gram-positive *Mycobacterium tuberculosis*, the extreme thermophile *Thermotoga maritima*, the specialized pathogens *Rickettsia prowazekii*, *Mycoplasma genitalium*, and *Mycoplasma pneumoniae*, the photosynthetic *Synechocystis* sp. strain PCC6803, and *Deinococcus radiodurans* (for a recent review, see reference 104).

Most bacteria contain several alternative σ factors belonging to the σ^{70} class, but two forms of σ^{54} rarely coexist in the same organism. That is, no more than one σ^{54} gene is usually found, the exceptions so far being *Bradyrhizobium japonicum*, *Rhodobacter sphaeroides*, and *Rhizobium etli* which each contain two *rpoN* genes encoding two σ^{54} proteins (24, 65, 82). Nonetheless, σ^{54}-RNA polymerase can be regulated independently at a wide variety of genes by virtue of a family of sequence-dependent enhancer proteins with promoter-specific binding sites (26). Each protein is controlled by its own signal transduction pathway, thus allowing a single σ^{54} polypeptide type to mediate transcriptional responses to a wide variety of physiological needs.

There is no obvious theme in the repertoire of functions carried out by the products of σ^{54}-dependent transcription. Among the proteobacteria, these include utilization of various nitrogen and carbon sources, energy metabolism (70), RNA

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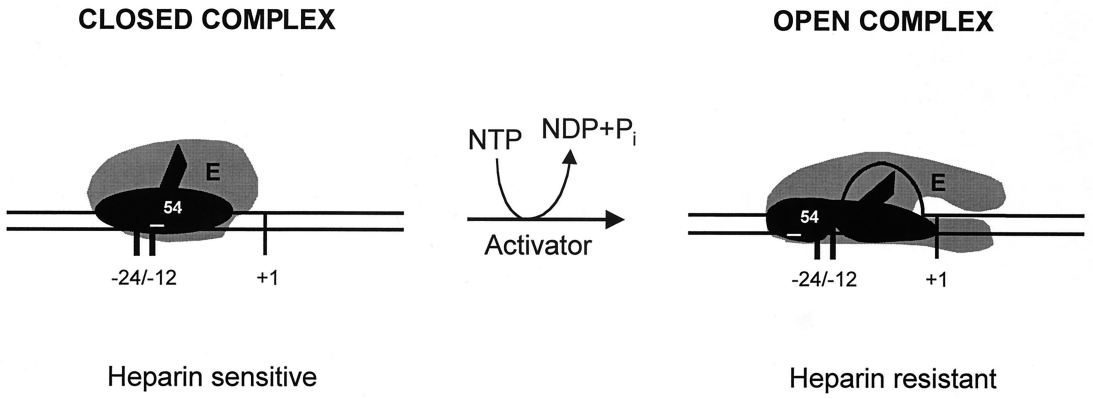

FIG. 1. RNA polymerase holoenzyme containing σ^{54} binds to −24 and −12 consensus promoters to form a stable closed complex which is transcriptionally silent. This complex can be disrupted by heparin in vitro. Activator-mediated nucleotide hydrolysis drives full open promoter complex formation. Open complexes are insensitive to heparin in vitro. NTP, nucleoside triphosphate; NDP, nucleoside diphosphate.

modification (44), chemotaxis, development, flagellation, electron transport, response to heat and phage shock (123), and expression of alternative σ factors (reviewed in references 1, 66, 80, and 104). It appears that σ^{54} is not usually essential for survival and growth under favorable conditions, except in *Myxococcus xanthus* (62).

The pattern of its occurrence in the bacterial domain argues for σ^{54} being biologically important and advantageous. Because initiation of transcription at a σ^{54}-dependent promoter absolutely requires the activity of the cognate activator protein, transcription can be very tightly regulated, with low levels of leaky expression (118). Moreover, the use of σ^{54} may confer one notable advantage: the capacity to vary transcriptional efficiency at a given promoter over a wide range without the use of a separate repressor. Genes transcribed by this form of polymerase can be silent or highly expressed (when activated), depending on the physiological or environmental conditions. Given these advantages, why then are relatively few bacterial genes transcribed by σ^{54}-RNA polymerase? The pathogenic *Neisseria* spp. seem to have abandoned the σ^{54}-RNA polymerase mode of transcription recently, their genomes still containing *rpoN* pseudogenes which have apparently undergone a deletion of the DNA-binding region (67). Presumably the main disadvantage of the σ^{54}-RNA polymerase mode of transcription is the requirement for significant stretches of intergenic DNA, and thus the need for larger chromosomes. The requirement for additional intergenic DNA arises from the DNA-looping mechanism of activator-RNA polymerase contact (5, 95). In order for cross talk between transcription units to be minimized, promoters would need to be well isolated from each other (73), as occurs in higher organisms where looping is common. In the case of *nif* genes in *Klebsiella pneumoniae* and *Azotobacter vinelandii*, their clustering could lead to promiscuous activation of one *nif* operon by the NifA bound to another *nif* operon's DNA. However, any cross activation would still be by the same signal transduction pathway, and might therefore be readily tolerated. Therefore, the same pressures that have led to the compactness characteristic of bacterial genomes may select against the increased use of σ^{54}-RNA polymerase.

CONTROL CIRCUITRY

σ^{54}-dependent activators bind to DNA sites at atypically long distances (for bacteria) from the start site for transcription, consistent with the looping mechanism of activation. This mechanism coexists with those regulating activity of the σ^{70}-holoenzyme, for which activators bind adjacent to the polymerase site and touch the enzyme without looping (45). The σ^{54}-holoenzyme forms a closed complex and occupies the promoter in this state prior to activation (97). This closed complex is unusually stable in the sense that it does not spontaneously isomerize into an open complex. Basal, unactivated transcription from the closed complex is intrinsically very low, consistent with the lack of repressors (see reference 26 but see reference 121 also) associated with σ^{54}-dependent promoters. This stable closed complex is a convenient target for the looping of activators bound to remote sites (106). At some promoters, the looping-out of the intervening DNA is facilitated by the bending protein integration host factor (IHF) (54). This effect can be mimicked by HU or even the mammalian nonhistone chromatin protein HMG-1 and can be bypassed by intrinsically curved DNA (13, 15, 92, 93). IHF has been proposed to stimulate recruitment of σ^{54}-polymerase at least one promoter (2). The outcome of looping is an activator-dependent isomerization of the closed complex into an open one, which leads to initiation of transcription. This mechanism is not observed for σ^{70}-holoenzyme, where both highly stable closed complexes and looping are rarely, if ever, used directly for activation (46). In essence, σ^{54} binding to RNA polymerase imposes a block on the initiation pathway whereby open-complex formation (DNA melting) can be controlled independently from closed-complex formation (DNA binding).

The use of enhancers, nucleotide hydrolysis for melting (120), and chromosome structure modification by bending are more commonly found in cases of eukaryotic polymerase II transcription than with bacterial σ^{70}-based transcription (reviewed in reference 64). Their involvement in bacterial σ^{54}-dependent transcription indicates that σ^{54} is responsible for significantly modifying the properties of the RNA polymerase to endow enhancer responsiveness. In this sense σ^{54} converts the polymerase to an enhancer-requiring enzyme (115). The mechanisms used by eukaryotic enhancers are not limited to the melting control observed in bacteria. The need for compactness in bacterial genomes may preclude the greater diversity of mechanisms that occur in mammalian cells.

As is the case for σ^{70}-holoenzyme, activation of σ^{54}-RNA polymerase occurs by signal transduction pathways using numerous and diverse activators (reviewed in reference 101). Although these pathways are diverse, they have a common terminal mechanism. In each case, the output appears to in-

volve the triggering of a hidden ATPase activity within an enhancer-binding activator protein. This ATPase is then used to overcome the block to DNA melting within the closed transcription complex and thereby allow transcription to initiate (122). When the physiological stimulus is removed, the activators no longer have their ATPase activities triggered and the cognate promoters revert to the inactive closed complex state. Many activators have their ATPase activities triggered by a phosphorylation cascade, as typified by the nitrogen regulator NtrC (124). Changing physiological conditions typically leads to phosphorylation of the protein's N terminus. Conformational changes then can lead to changes in activator affinity for enhancer DNA sites, multimerization on the DNA, and most important, assembly of a DNA-bound complex with ATPase activity (96). The ATPase activity is within the central domain of the activator, which may adopt a fold common to other purine nucleotide-binding and hydrolyzing proteins, and is predicted to show some structural similarity to members of the AAA+ protein family to which it belongs (87). Our understanding of NtrC is increasing with knowledge of its N- and C-terminal domain structures and how its activities are controlled by phosphorelay (61, 90, 91). Many other activators, e.g., NifA and PspF, do not rely on phosphorylation but instead react with small-molecule effectors or inhibitory polypeptides (e.g., references 32, 34, 101 and 129).

Additional physiological controls may be superimposed on the activator-dependent control of transcription from σ^{54}-dependent promoters. For example, the *pseudomonas putida Pu* and *Po* promoters and the *Rhizobium meliloti dct* promoter are subject to catabolic repression by mechanisms which appear to be independent of signal transduction via the cognate activators (19–21, 33, 75, 83, 121). RNA polymerase holoenzyme at these promoters may also be regulated by a mechanism involving ppGpp (e.g., reference 110). Furthermore, the FtsH (HflB) protease is required for full σ^{54} activity in vivo at least at some promoters (14).

Although expression of σ^{54} is constitutive in many bacteria investigated (reviewed in reference 128), it is temporally regulated in *Caulobacter crescentus* (3) and *Chlamydia trachomatis* (77). Transcription of *rpoN*, encoding σ^{54}, is probably subject to negative autoregulation in several bacteria including *Acinetobacter calcoaceticus* (35), *Azotobacter vinelandii* (79), *B. japonicum* (65), *K. pneumoniae* (47, 78), *P. putida* (63), and *R. etli* (82, 83).

The paradigm for enhancer-dependent transcription in eubacteria is characterized by interaction between σ^{54}-RNA polymerase and an activator of the NtrC/NifA family. However, evidence is emerging that there may be more-complex assemblies of protein at some promoters controlled by σ^{54} that contribute to sophisticated regulatory responses. For example, cyclic AMP receptor protein mediates repression at the *dctA* promoter of *Sinorhizobium meliloti* (121).

A further example of complex control of σ^{54}-dependent transcription may occur in *B. subtilis* where the AhrC protein represses arginine biosynthesis by binding to operator sites in the promoter regions of arginine biosynthetic genes (71, 84). An AhrC-binding site is located between the genes for glutamate dehydrogenase (*rocG*) and 1-pyrroline 5-carboxylate dehydrogenase (*rocA*), both of which may be under the control of σ^{54}-RNA polymerase and the activator RocR (43). AhrC footprints a region directly adjacent to the *rocA* gene (nucleotides −22 to −2), suggesting the possibility of a regulatory interaction with σ^{54}-RNA polymerase.

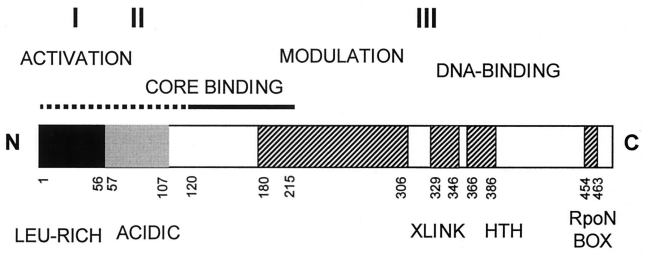

FIG. 2. Domain organization of the σ^{54} protein. *E. coli* σ^{54} (amino acids 1 to 477) can be divided into three regions (I to III [80]). DNA-binding functions (127) and associated motifs (DNA cross-linking [XLINK] region [7], helix-turn-helix [HTH] motif [81], and RpoN box [111]) reside in the C terminus, adjacent to sequences that modulate DNA binding (amino acids 180 to 306) (12). Activator responsiveness involves region I sequences (10, 98, 109), and region II is acid and variable. The main core binding determinant from amino acids 120 to 215 (40, 127) is shown. A full σ^{54} amino acid alignment of known sequences is available at http://www.bio.ic.ac.uk/staff/mbuck/alignment.rtf.

σ^{54} DOMAIN STRUCTURE, PROMOTER RECOGNITION, AND CORE INTERACTIONS

Sequence alignments, mutation analyses, and protein fragmentation studies have led to a picture of the overall domain structure of σ^{54}, mainly using the proteins from enteric bacteria (11, 40, 79, 98, 127). σ^{54} was divided into three regions by sequence conservation (Fig. 2) (80). Primary DNA-binding functions (127) are in region III with a DNA cross-linking region (7) and associated motifs, the helix-turn-helix motif (81) and RpoN box (111) near the C terminus. Adjacent sequences modulate this activity (12) and others constitute the minimal core-binding domain (40, 113). Region II is variable and in some species, such as *Rhodobacter capsulatus*, is almost completely absent. In many (but not all) bacteria, region II is acidic, and it has been implicated in DNA melting (126), in the transition from the closed to open complex (E. Southern and M. Merrick, submitted for publication), and in assisting σ^{54} binding to homoduplex and heteroduplex DNA (8). The lack of conservation of region II suggests that none of its activities are essential. The amino-terminal 50 residues (region I) comprise a domain that performs two distinct functions: (i) inhibiting polymerase isomerization and initiation in the absence of activation (10, 109, 115) and (ii) stimulating initiation in response to activation (98, 109). It is now clear that there is considerable cross talk among these domains for the full function of σ^{54}.

DNA binding. The primary DNA-binding activity for recognition of double-stranded promoter DNA resides in a C-terminal domain (7, 50, 81, 98, 111, 127). Mutations within this region eliminate such binding. Unlike σ^{70}, the DNA-binding activity of σ^{54} is not fully masked and σ^{54} is able to bind to certain promoters in the absence of core polymerase (4). Nonetheless, holoenzyme binds tighter than does σ^{54} alone. Two other regions can affect the affinity of binding: the N terminus plays complex multiple roles in binding to both duplex and melted DNA (10, 17, 41, 52, 59, 60, 98, 117), and the segment between the C terminus and the major core-binding determinant influences binding affinity (12, 50). These regions do not necessarily make direct DNA contact.

The σ^{54} promoter recognition sequence includes short elements at nucleotides −12 and −24 (85) with extensive conservation in between these two (1, 118). Mutant analyses suggest that the −24 element makes the greater contribution to binding, an argument based on the ability of mutant complexes to retain −24 contacts while losing −12 region contacts; no complex has been found that retains only −12 region contacts (55,

127). Remarkably, the subdomains that recognize these elements are not yet definitively identified. Recognition of the −12 sequence appears to be very complex (41, 55, 81, 86, 98, 118, 119, 127). It was initially proposed to involve a C-terminal potential helix-turn-helix motif (27, 81) and the N terminus (98). It is likely that −12 recognition is accomplished by a structure contributed by more than one region of the protein (22; L. Wang and J. D. Gralla, unpublished data). The −24 recognition is presumed to occur via the C terminus (50, 98, 127), although direct evidence is still lacking. The highly conserved RpoN box (111) is a candidate for this interaction.

There is considerable variation in the DNA sequences of σ^{54}-dependent promoters; as is the case for σ^{70} promoters, virtually all sequences deviate from the consensus sequence (1). The artificial introduction of consensus nucleotides generally increases binding, but transcription is not increased in all cases (25). Some sequence changes lead to detectable levels of leaky transcription, apparently through defects in the use of the −12 element (118, 119). It appears that a balance must be struck between promoter occupancy, transcription levels, and prevention of unregulated transcription. The affinity of the closed complex can be high enough so that promoters are occupied in vivo prior to activation. The number of σ^{54} molecules in *Escherichia coli* cells is close to 100 (compared to 600 to 700 of σ^{70} [57]) which is greater than the number of promoters (less than 20 in the *E. coli* genome). Thus, even low-affinity promoters may be significantly occupied prior to activation. This seems likely given the apparent lack of promoter recruitment of the σ^{54}-RNA polymerase holoenzyme by its activators (95, 121).

Core polymerase binding. The interface between σ^{54} and core RNA polymerase is probably very extensive. Mutations in the central region of σ^{54} eliminate binding to core (56, 112, 113, 127). Additional contributions come from the C-terminal DNA-binding domain and N-terminal region I (16, 17, 40). Although σ^{54} is not related to σ^{70} by primary sequence, it binds to the same core polymerase, likely with similar affinity (40, 99). However, there is a short region of potential similarity in the central domain (112) and small-angle X-ray-scattering studies suggest that the core-binding domains of σ^{54} and σ^{70} have similar shapes (107). Results with tethered iron chelate methodology have suggested related binding arrangements (125). The core-binding interface of σ^{70} also involves widely separated regions of that protein (100), yet another point of similarity between σ^{54} and σ^{70}. σ^{70} has determinants of interaction with the core and with the DNA in close proximity. The N terminus of σ^{54} also has these properties, which may reflect a coordinated behavior of domains during transcription initiation.

Indeed, the σ^{54}-core interface appears to change during the transcription cycle. After initial binding, there is a slow conformational change in the holoenzyme, suggestive of a stabilization of σ^{54}-core interactions (99). In initiated complexes, the N-terminal region I conformation appears to have changed with respect to closed complexes (16). This region is central to the control of DNA melting (127). The interaction of region I with core likely contributes some of the specialized properties of the holoenzyme, including its ability to be controlled at the DNA melting step (9, 10, 40, 41).

Some sequences in the DNA-binding domain of σ^{54} appear to be required for the activator-independent heparin stability of the holoenzyme on early melted DNA, suggesting that they contribute to the interface of σ^{54} that interacts with core (88, 89; M. Pitt and M. Buck, unpublished data). The functioning of this interface may be important during the early stages of DNA opening. The existence of the interface was suggested by protein footprint experiments (16, 17).

MECHANISM OF PROMOTER ACTIVATION

The key activation event is the opening of the DNA within the closed complex of σ^{54} holoenzyme at the promoter. This is accomplished using the ATPase activity of the activator, which loops from the enhancer site (96). The interactions between activator and the σ^{54}-RNA polymerase holoenzyme appear to be transient, and the only direct physical evidence has been by a cross-linking assay using the DctD activator (60, 69).

As activators of the σ^{54}-holoenzyme are related to the purine nucleotide-binding and hydrolyzing proteins, they would be expected to use the ATPase to bring about conformational changes. It is not known how the proposed energy coupling impacts upon the holoenzyme to cause it to open the DNA. Sequence analysis of activators does not reveal obvious similarity to known helicases (96, 122).

Clues from deregulated transcription. One view of the activation process is that σ^{54} organizes the holoenzyme so that it cannot fully melt DNA; the activator then overcomes this block. In this view, mutants that allow unregulated melting provide valuable clues to the mechanism of activation (115). Such mutants have been found in both the σ^{54} protein and in the DNA sequence of the promoter.

Deregulated σ^{54} mutants, allowing activator-independent transcription, map predominantly in the N-terminal region I and in a very limited set of sites within the C-terminal DNA-binding domain (18, 22, 108, 109, 115–117; Wang and Gralla, unpublished). Mutant promoters that allow activator independent transcription have in common a substitution for the consensus C at nucleotide −12 (118, 119). These locations and other data concerning −12 region recognition have led to the idea that there may be a complex molecular structure, involving the protein's N and C termini and the promoter −12 region, that keeps the DNA firmly closed (Fig. 3) (9, 22, 41, 52, 60; Y. Guo, C. M. Lew, and J. D. Gralla, submitted for publication).

In order to understand how DNA melting originates and propagates, various studies have used DNA probes that attempt to mimic intermediates along the melting pathway. Initial studies showed that transcription of preopened promoter DNA heteroduplexes did not bypass the activator requirement (10, 122). This suggests that conformational changes in the protein must be required in addition to those in the DNA (10). More-recent studies confirmed this but showed that heteroduplexes that keep nontemplate position −11 firmly double stranded could be transcribed without activator (Guo, Lew, and Gralla, submitted). Certain DNA probes that mimic the critically important upstream −11 fork junction of the transcription bubble (51) bind both isolated σ^{54} and holoenzyme exceptionally tightly, using the template strand of the fork (9, 41, 52; W. Cannon and M. Buck, unpublished data). This binding is also inhibited by exposure of the nontemplate position −11 (52). These observations suggest that interactions near −11 are critical, perhaps in controlling the required conformational changes in the holoenzyme (Fig. 3) (Guo, Lew, and Gralla, submitted).

Deregulated σ^{54} mutants lose tight DNA binding to a variety of fork and heteroduplex probes (9, 41, 52, 59, 60; M. Chaney and M. Buck, unpublished data), particularly when the fork junction is at −11 (52). This is true whether the deregulation mutation is in the N or C terminus of σ^{54} or in the −12 region of the promoter. Deregulated mutants gain the ability to bind more tightly to downstream structures containing melted DNA

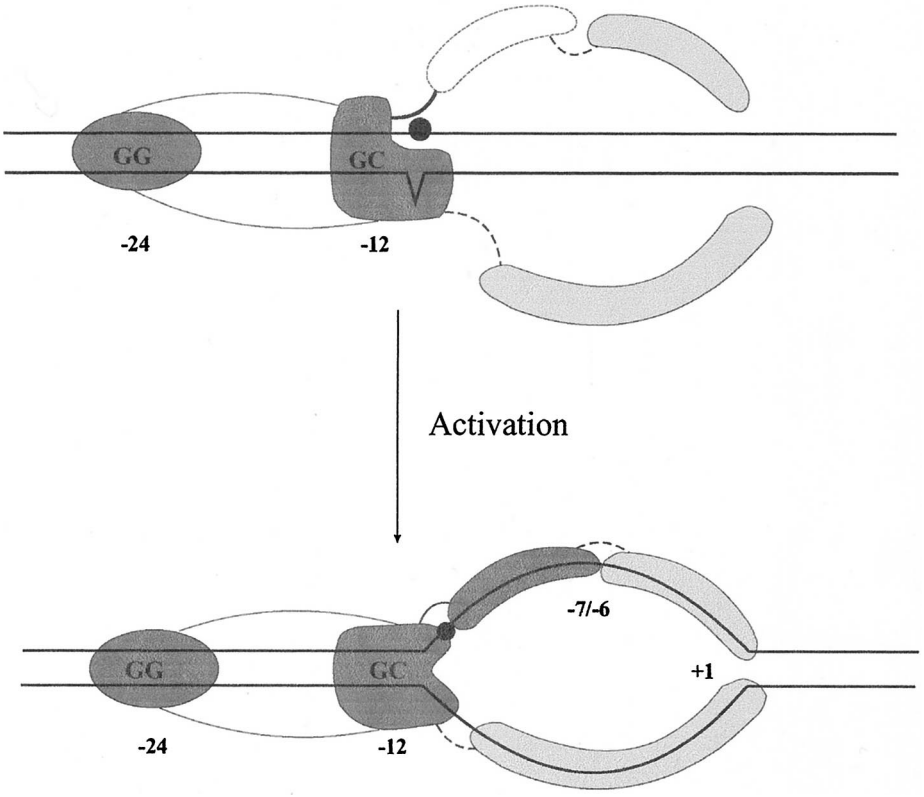

FIG. 3. Cartoon model showing activities proposed for activation via DNA melting. Dark shading indicates activities primarily in σ^{54}. These activities include duplex (double-stranded DNA) binding at nucleotides −24 and −12 and fork junction binding. The −7 to −11 single-strand binding activity is seen only after activation. *Light shading* indicates single-strand binding activities contributed by RNA polymerase holoenzyme. The solid circle represents the −11 connector nucleotide that inhibits the spread of unactivated melting when unpaired. (Top) Prior to activation, the holoenzyme is stabilized on the DNA using primary interactions at nucleotide −24. The unactivated complex includes a form shown here in which a molecular structure near −12 contributes to binding and prevents the spread of early DNA melting to downstream positions. The N terminus and the fork junction connector position −11 (solid circle) are key determinants in locking the system in a closed state. Deregulated bypass mutations destroy this σ^{54}-DNA structure at −11 and allow partial engagement of the downstream single-strand binding activities; these are shown downstream of −12 and above and below the DNA in an unengaged state. (Bottom) Upon activation, ATP triggers conformational changes in σ^{54} at the fork junction that involve the σ^{54} N terminus. The upstream nontemplate strand binding activity is exposed, and interactions through the connector can now be established. The single-strand DNA-binding activities stabilize the spread of melting.

(10, 18, 52, 119; Wang and Gralla, unpublished). Thus, one aspect of regulation is to prevent this. In closed complexes, σ^{54} contributes to the maintenance of a very local DNA distortion that has all the signatures of local DNA opening and with which a fork junction structure must be associated. If base pair −11 is transiently melted by wild-type holoenzyme (Fig. 3), a tight fork junction complex would be created along the template strand. This would not propagate melting due to the exposure of the inhibitory nontemplate (52; Guo, Lew, and Gralla, submitted). Thus, inappropriate downstream opening would be prevented.

The nature of the interaction at this upstream fork junction changes during activation, as indicated by altered sensitivity of the DNA to chemical probes and altered patterns of protein-DNA cross-linking, suggesting that the activator overcomes this inhibition of melting (9, 11, 85, 86, 94; Guo, Lew, and Gralla, submitted). The N terminus is centrally involved in these changes as suggested by the results of experiments with a σ^{54} in which this region had been deleted (10, 42, 52, 127). Activator can no longer function, confirming that region I has a positive role in activation (109, 117). With this mutant, one can supply the missing region in *trans* and restore tight binding to heteroduplex probes (9, 10, 42). Thus, one view is that the N-terminal region I is part of a molecular switch (52) that helps keep melting in check within closed complexes but upon activation switches its interactions to a new set that contributes to open-complex formation (Fig. 3) (9, 10, 41, 42; Guo, Lew, and Gralla, submitted).

Role of the σ^{54} polypeptide. Various results underscore the pivotal role of σ^{54} in activation and DNA melting by holoenzyme and suggest that it may be the primary target of the activator. σ^{54} has the specificity that recognizes the DNA at the upstream fork junction (52). σ^{54} bound to heteroduplex DNA probes containing such junctions changes conformation independently of core polymerase in a reaction that requires activator and nucleotide hydrolysis (9). In the isomerized complex, σ^{54} interactions give an extended DNase I footprint that approaches the start site and some DNA melting occurs over this region, (W. Cannon, M.-T. Gallegos, and M. Buck, unpublished data). These considerations indicate that conformational changes in σ^{54} itself are likely to be very early events triggered by activators.

These activator-driven conformational changes in σ^{54} are associated with conformational changes in the holoenzyme. The DNA cross-linking pattern of σ^{54} in holoenzyme is altered upon activation, and this and other changes occur specifically at the upstream fork junction (Guo, Lew, and Gralla, submitted). The new cross-link requires activator and ATP and establishes interactions with the melted nontemplate strand segment adjacent to the upstream fork junction. Related changes

have been detected during melting by σ^{70}-holoenzyme, which also has an inhibitory nucleotide (Fig. 3) separating the fork junction and nontemplate strand interactions (51; Guo, Lew, and Gralla, submitted). The emerging view is that the two melting pathways (σ^{70} and σ^{54}) may be similar with the fundamental distinction that σ^{54} organizes the holoenzyme so that its conformational changes are firmly prevented in the absence of activator. The N-terminal region I of σ^{54} plays a key role in this process.

Bacterial promoter melting relies on activities that engage the single-stranded DNA within the open complex (51, 52, 76; Guo, Lew, and Gralla, submitted). Although region I controls melting, the activities likely lie in several parts of σ^{54} and probably in core as well. Both σ^{54} and σ^{70}-holoenzymes have activities that bind upstream fork junctions and activities that bind the single-stranded DNA (51, 52). In the case of σ^{70}-holoenzyme, a series of two conformational changes occurs that allows these activities to work together to fully engage the melted DNA (Guo, Lew, and Gralla, submitted). In σ^{54}-holoenzyme, the engagement of the downstream single-stranded DNA can lead to transient transcription in vitro but this is promiscuous, as evidenced by its sensitivity to heparin (10, 114, 116, 117). One view is that σ^{54} organizes the holoenzyme into a closed form that cannot engage the full DNA bubble from upstream fork to start site, which would need to propagate through the −11 inhibitory nucleotide. The N-terminal region I would have central involvement in keeping the enzyme closed and in responding to activator to change conformation to open the enzyme and hence the DNA (9, 10; Guo, Lew, and Gralla, submitted; Cannon, Gallegos, and Buck, unpublished data).

FUTURE DIRECTIONS

Remaining problems include determining the precise sites of contact for activators in σ^{54}-holoenzyme, working out how the activator uses ATP to achieve conformational changes, and determining what these conformational changes are and how they are used. Some answers will depend upon structural information and biophysical approaches. Genetic methods still continue to be a way forward (e.g., reference 47). We note that the σ^{54} transcription mechanism complements the common bacterial mechanism with a dependence on enhancers and ATP hydrolysis for initiation that is like mammalian RNA polymerase II. In a sense, this bacterial arrangement mimics the specialization in eukaryotes where different polymerases have differing dependencies on enhancers and ATP hydrolysis. It will be a challenge to work out the advantages of each type of mechanism and place them together in evolutionary context.

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